EXHIBIT A

2/15/00

~~Clean DNA first.~~

~~Run a gel~~

~~there are just a~~

~~little DNA in expect size~~

~~So. improve Digest~~

Digest

DNA 40 ul buffer 10 ul
H_2O 42.5 ul EcoRI 8.5 ul } overnight 37°C

[illegible] [illegible] Feb 18 2000
GENOSYS [illegible]
HAPDAMP[illegible]
5'-[illegible]
[illegible] | Tm=[illegible]°C
[illegible] | [illegible]
[illegible] | MW=[illegible]

[illegible] [illegible] Feb 18 2000
GENOSYS [illegible]
HAPDAMP2
5'-TGTGCCGTTTGGTACCTATA
7.300 | Tm=[illegible]°C
[illegible] | [illegible]
[illegible] | MW=[illegible]

→ primer

3 [illegible] Feb 18 2000
GENOSYS [illegible]
HAPDAMP2
5'-TGTGCCGTTTGGTACCTATA
7.200 | Tm=[illegible]°C
235.[illegible] | [illegible]
38.[illegible]mol | MW=[illegible]

→ New

2/17/00

Digest – to ~~check~~ differential digest procedure for dam gene detection

11039 or D153 DNA 1 ul

10 x buffer 1 ul

H_2O 7.5 ul

enzyme 0.5 ul
↓
3 digest Sau3A I Dpn I Mbo I
↓ ↓
both unmythlased gene do-mythlased gene

Digest 37°C 4 hr.

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[signature]
Signed

Signed

Inverse PCR procedure

Use 100 ng EcoRI digest set up 3 ligations

1039 EcoRI cut DNA	1	2	5
10× buffer	5	5	5
T4 ligase (NEB)	0.5	0.5	0.5
H_2O	33.5	33.5	33.5
	50 ml		

16°C overnight ; heat inactivate

for PCR

2+1 100

Run a gel to detect differential digest procedure for dam gene.

Ladder ① 1039 Sau3AI ③ 1039 MboI ④ 1039 DpnI

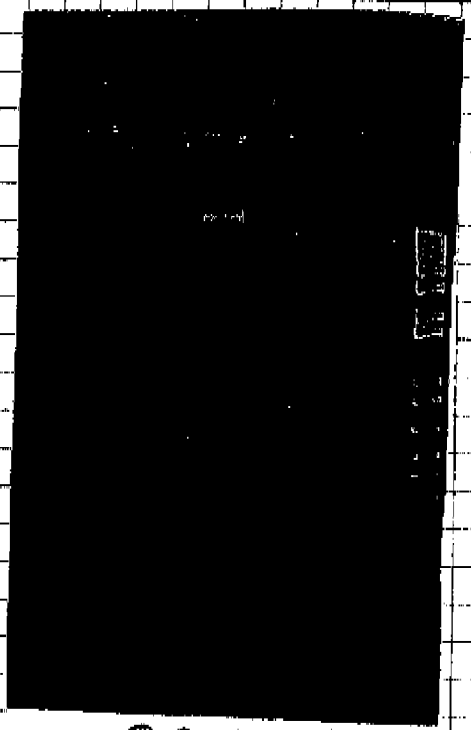
② D153 ⑤ D153 ⑥ D153

there once some is dam gene in D153.

Sau3AI → digest GATC both mathyled and unm...

MboI → digest GATC only un....

DpnI → digest GATC only mathylated.



⑥ ⑤ ④ ③ ② ①

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